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ANTI-INFLAMMATORY LIPID MEDIATOR LIPOXIN A4 PRODUCTION FOLLOWING MYELOABLATIVE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Systemic inflammation results from tissue damage incurred from the conditioning regimen and contributes to early post-allogeneic HSCT complications such as aGVHD. Mechanisms that actively govern inflammation resolution and dampen exaggerated inflammatory responses have recently been discovered, but have not been characterized after allogeneic HSCT. Potent anti-inflammatory / pro-resolving eicosanoid lipid mediators play a pivotal role in this process. Lipoxin A4 (LxA4) is one such lipid mediator derived from arachidonic acid. In nanomolar concentrations, LxA4 actively inhibits the synthesis and action of a number of Th1 cytokines known to be important in aGVHD, such as TNFα and IL-1. We wished to determine if and when LxA4 is produced following allogeneic HSCT.
Methods: Plasma from 16 patients (15-55 years, mean age 37 years) undergoing allogeneic HSCT was taken at 5 sequential time points (median, +/− 2 days): Pre-conditioning (day -8), Early 1 (day +4), Early 2 (day +15), Early 3 (day +28), and Late 1 (day +52). All conditioning regimens were myeloablative and included Bu/Cy (n = 5), Bu/Flu/Thymoglobulin +/− TBI (n = 5), or Cy/TBI +/− VP16 or thiotepa (n = 6). Age and sex-matched healthy volunteers (n = 16) served as controls. LxA4 was extracted and quantitatively measured by competitive ELISA according to standard procedures (Oxford Biomedical).
Results: Plasma LxA4 for controls and patients is presented below (Table). Bonferoni-corrected t test comparisons between the control group and each of the 5 time points (Pre-conditioning to Late 1) identified mean LxA4 concentration in the patients was significantly higher compared to the control group at all three time points in the first month after allogeneic HSCT ($p < 0.01$). Univariate repeated measures ANOVA identified a difference in the mean LxA4 concentration in the patients over time from Pre-conditioning until the Late 1 time point ($F = 2.88$, $P = 0.0016$).
Conclusions: LxA4 is produced systemically in the first month following myeloablative allogeneic HSCT and peaks approximately two weeks post-conditioning. Analyses are underway to determine whether there is a correlation between LxA4 levels and early post-allogeneic HSCT complications. This study provides insight into endogenous mechanisms that may promote the resolution of inflammation post-transplant, and may lead to the development of novel therapies (e.g. aspirin-triggered lipoxin analogues) aimed at reducing systemic inflammation.

Table 1. Lipoxin A4 concentrations in plasma.

	Controls	Pre-Conditioning	Early 1	Early 2	Early 3	Late 1
Mean Plasma LxA4 (ng/mL)	0.163	0.274	0.387 *	0.432 *	0.324 *	0.157
Standard Deviation	0.143	0.075	0.182	0.231	0.134	0.074
Range (ng/mL)	0.000-0.525	0.162-0.411	0.147-0.818	0.152-0.934	0.123-0.554	0.110-0.345

*p<0.01 versus controls.

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NONINVASIVE PREDICTION OF GRAFT-VERUS-HOST DISEASE FOLLOWING ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION BY GENE EXPRESSION PROFILING

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Background: Chronic graft-versus-host disease (cGvHD) and immunosuppressive medications for its treatment result in significant morbidity and mortality, limiting the benefit of allogeneic hematopoietic cell transplantation (HCT). A noninvasive, diagnostic and prognostic test is needed to determine which patients have active cGvHD at present and which subset will have active cGvHD in the future.
Methods: We performed peripheral blood gene expression profiling to identify transcriptional biomarkers that discriminate active cGvHD requiring immunosuppression. 63 patients (median age 50 yrs;19-64) following allogeneic HCT (median 475 days post) on an IRB approved protocol. Samples were randomly selected into a training set (23 cGvHD/19 without cGvHD) and test set (12 cGvHD/9 without cGvHD) and processed on the Agilent platform. AILUN, GeneSpring, SAM, and PAM were used to select a minimum gene-set (FDR < 5%) to predict cGvHD in the training set. Patient characteristics in the two groups were collected for confounder/interaction analysis across the following parameters: age, gender, disease histology, disease status at HCT, graft source, conditioning regimen, white blood count (WBC) at sample, and follow-up status including relapse and survival. Type, grade, and activity of acute GvHD and cGvHD at time of sample and at most recent follow-up were determined by two independent investigators by standard clinical criteria.
Results: There is considerable heterogeneity among cGvHD patients, not attributable to confounding factors, suggesting molecular heterogeneity in the development and progression of cGvHD. A minimum of 10 unique genes selected from SAM and PAM predict active cGvHD at the time of the sample with 95% sensitivity and 78% specificity in the training set, and 75% sensitivity and 78% specificity in the test set. Eight and four of ten genes are enriched in NK and CD4 T cells, respectively, and involved in the IL-10 signaling pathway ($p < 0.01$). The 10 gene-set predicted the active cGvHD at last follow-up (median 849 days post) with 85% sensitivity and 83% specificity.
Conclusion: Peripheral blood gene-expression across a 10 gene-set shows strong correlation of the predictive probability of cGvHD. Given reasonable diagnostic accuracy and sensitivity for prediction of future cGvHD, additional longitudinal studies of this cohort and a prospective cohort are underway to further improve the accuracy of this approach and predictive power of this gene-set.

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INTEGRIN αV INHIBITION REDUCES GVHD BY TARGETING INFLAMMATORY NEOVASCULARIZATION

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Integrins are receptors that mediate cell signaling, cell mobility, extracellular matrix (ECM) interaction and cell cycle regulation. The integrin αvβ3 is a receptor that regulates cell adhesion to ECM, which is a prerequisit for endothelial cells (EC) during vascularization. We therefore hypothesized that the integrin αvβ3 may be required for neovascularization as a feature of inflammation and for the migration of immune cells which both could be relevant during graft-versus-host (GvHD) development.

By using radionucleotide labeled integrin αvβ3 ligand and positron emission tomography (PET) imaging we could demonstrate increased integrin αvβ3 expression in the gastrointestinal tract (GIT) following murine allogeneic hematopoietic cell transplantation (alloHCT) when GvHD evolved. Pharmacological inhibition of αvβ3 integrin abrogated the αvβ3 integrin signal in the GIT detected by PET. Reduced αvβ3 integrin expression translated into improved survival of mice developing GvHD and less severe GvHD histopathology score of the GIT. Also 18F-Fluordesoxyglucose-PET demonstrated a lower signal intensity in the GIT when pharmacological inhibition of integrin αvβ3 was applied, indicative for reduce inflammatory activity. Although we observed increased integrin αv expression on donor T cells, the protective effect of integrin αv inhibition was not dependent on lower donor T cell proliferation or abnormal migration as assessed with bioluminescence imaging. Histological analysis demonstrated inflammatory neovascularization in the GIT tissue in BMT recipients that developed GvHD.

These data indicate that αvβ3 integrin is a novel target to interfere with GvHD based on inhibition of inflammatory neovascularization.